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Antidepressant effects of TNF-α blockade in an animal model of depression

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ABSTRACT

Pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) have repeatedly been shown to play a pivotal role in the pathophysiology of depression. Therefore, we tested the possible antidepressant-like effect of the anti-TNF-α drug etanercept in an animal model of chronic mild stress. Male Wistar rats were assigned to a non-restrained and a restrained protocol for 5 weeks. From beginning of the third week the animals were treated either with Ringer solution daily or with etanercept twice a week (0.3 mg/kg, i.p.) instead of Ringer solution ($n = 12$ each). As reference, imipramine (10 mg/kg, i.p.) was administered in a third restraint group daily. Naïve non-treated non-restrained rats served as healthy controls ($n = 12$). In the forced swim test (FST) depression-like behaviour induced by restraint was recorded as enhanced immobile time and reduced climbing activity of the vehicle-treated group in comparison to the naïve and the non-restrained vehicle treated group. The treatment with etanercept significantly reduced the depression-like effects resulting in reduced immobile time in the FST and intensified climbing behaviour ($p < 0.01$, $p < 0.05$), both similar to the antidepressive-like effect of imipramine ($p < 0.01$ both). The repeated restraint induced a loss of body weight gain in the Ringer-treated group which was not reversed, neither by imipramine nor by etanercept. The antidepressant effects of blocking TNF-α using etanercept may be caused by enhancement of serotonergic or noradrenergic neurotransmission or normalization of stress hormone secretion which has to be substantiated in further studies.

1. Introduction

Changes regarding the immune system and specifically the cytokine system – in which tumour necrosis factor-alpha (TNF-α) is a pro-inflammatory key signalling molecule – have been shown to be involved in the development of psychiatric disorders (Himmerich et al., 2009). Especially, TNF-α might contribute to the pathogenesis of depression, because plasma levels of TNF-α and its soluble receptors have been found to be elevated in acutely depressed patients (Himmerich et al., 2008), and experimental stimulation of TNF-α production leads to depression-like emotional and cognitive disturbances in humans (Reichenberg et al., 2001).

It has been postulated that the activation of the cytokine system might play a causative role in the depression-related activation of the hypothalamic–pituitary–adrenal (HPA) system (Maes et al., 1993). Experimental studies applying immune stimulation in humans (Reichenberg et al., 2001, 2002) as well as in rodents (Linthorst and Reul, 1999; Pollak and Yirmiya, 2002; Engler et al., 2011) support the view that inflammatory cytokines are causally involved in the neuroendocrinological and behavioural alterations of patients with depressive disorders.

As pro-inflammatory cytokines and serotonergic homeostasis have both been implicated in the pathophysiology of depression, Zhu et al. hypothesized that cytokines might activate neuronal serotonin transporters. This idea underlines the theory of a serotonin deficiency during depression and the pharmacodynamic mechanism of selective serotonin reuptake inhibitors (SSRI) in the treatment of depression, because SSRIs lead to recovery from depression via deactivation of serotonin transporters. Indeed, Zhu et al. found TNF-α stimulated serotonin uptake in both a rat embryonic raphe cell line and in mouse midbrain and striatal synaptosomes. These results provide evidence that pro-inflammatory cytokines can acutely regulate neuronal serotonin transporter activity. A mitogen-activated protein kinase may be involved in

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this mechanism (Zhu et al., 2006; Himmerich and Sheldrick, 2010). Moreover, pro-inflammatory cytokines such as TNF-α affect the tryptophan metabolism directly or indirectly by stimulating the enzyme indoleamine 2,3-dioxygenase, which leads to a peripheral depletion of tryptophan (Wichers and Maes, 2002). The aromatic amino acid tryptophan functions as a precursor for the monoamine neurotransmitter serotonin in the brain. In other psychiatric disorders, for example narcolepsy, it has been hypothesized that TNF-α might also contribute to the destruction of neurons (Himmerich et al., 2006, 2009; Lotrich, 2012; Raison and Miller, 2011; Müller and Schwarz, 2007). Cytokines may additionally influence glutamatergic signalling. For example, interleukin (IL)-18 impairs long term potentiation and glutamatergic neurotransmission (Curran and O'Connor, 2001); however, it is not yet clear, whether TNF-α might also act on glutamatergic signalling.

It has been postulated on the basis of in-vitro as well as in-vivo studies that the therapeutic action of antidepressants may be partially caused by their influence on cytokine production in addition to their direct effect on monoaminergic neurotransmission. Antidepressants have been, for example, shown to decrease IL-1β (Himmerich et al., 2010b), interferon (IFN)-γ (Himmerich et al., 2010a) and TNF-α production (Ignatowski et al., 1996; Nickola et al., 2001). Moreover, TNF-α has been shown to modulate norepinephrine (NE) signalling. For instance, it inhibits the release of NE in hypothalamic and hippocampal slices from rats (Reynolds et al., 2005; Elenkov et al., 1992). However, after chronic administration of the antidepressant drug desipramine, a NE reuptake blocker and active metabolite of imipramine, to rats, TNF-α no longer inhibited but rather facilitated NE release from electrically depolarized hippocampal slices (Reynolds et al., 2005). Therefore, we have to assume that modulation of TNF-α signalling may be one of the mechanisms how antidepressants work.

Taken together, pro-inflammatory cytokines like TNF-α might exert their depressogenic effects by an activation of the HPA axis, an activation of neuronal serotonin transporters, the stimulation of the indoleamine 2,3-dioxygenase, by immunologically mediated destruction of neurons, and the release of glutamate. Antidepressants, in turn, may unfold their therapeutic action in part by modulating the cytokine system. Hence, reducing the pro-inflammatory cytokine production could be a possible mechanism of antidepressants (Himmerich et al., 2010a, 2010b).

Moreover, anti-TNF-α-agents such as etanercept, a human TNF-α receptor p75-Fc fusion protein, have been shown to exert antidepressant effects in patients with moderate to severe psoriasis (Tyring et al., 2006). Therefore, blocking TNF-α might be a novel strategy against depression which should be tested in an animal paradigm.

Within the immune system, TNF-α is a cytokine that is involved in the development and maintenance of the immune response. It is a pivotal cytokine in inflammation, and its critical role has been demonstrated in a number of diseases such as rheumatoid arthritis, ankylosing spondylitis, Crohn's disease and psoriasis. Etanercept binds to TNF-α, thereby blocking its interaction with cell surface receptors and attenuating its pro-inflammatory effects (Zhou, 2005). Etanercept is approved and generally well-tolerated for the treatment of immune-mediated inflammatory conditions including rheumatoid arthritis, juvenile idiopathic arthritis, psoriasis, psoriatic arthritis and ankylosing spondylitis.

In humans, etanercept is absorbed slowly from the site of subcutaneous injection, with time to peak concentration at approximately 48–60 h, and is cleared slowly from the body with a half-life period of 70–100 h. The absolute bioavailability of etanercept is about 58% in healthy subjects following subcutaneous administration (Zhou, 2005).

In the reported experiment we tested the effects of etanercept and imipramine on stress-induced depression-like behaviour in rats. Imipramine, in contrast to etanercept, has a much shorter half-life period compared to etanercept. The half-life of imipramine is between 9 and 20 h (Ciraulo et al., 1988). Therefore, imipramine is taken about one to three times daily for antidepressant treatment in humans, whereas etanercept is injected subcutaneously twice a week. Accordingly, we applied imipramine daily and etanercept twice a week in the current investigation. However, to our knowledge, pharmacokinetic data of etanercept are not available in rats.

In rats or mice, repeated restraint stress induces repeated transient elevations of plasma corticosterone (Strausbaugh et al., 1999) and subsequent reduction of glucocorticoid receptor expression (Chiba et al., 2012). This form of chronic mild stress also evokes depression-like behavioural changes accompanied by working memory and learning deficits (Albonetti and Farabollini, 1993; Beck and Luine, 2002; Regenthal et al., 2009) and appears therefore to be an appropriate model for depression. In order to test antidepressant effects, the forced swim test (FST) has a high predictive value for the efficacy of drugs in antidepressant therapies. Originally, the reduction of immobile time in an inescapable water basin in favour of climbing (escape) behaviour and active swimming after acute antidepressant administration was measured. Furthermore, the FST serves as a marker for the behavioural state associated with depression-like symptoms (Kitada et al., 1981; Cryan et al., 2005; Porsolt et al., 1979).

We hypothesized, that etanercept given in a subchronic treatment regime could be beneficial for the behavioural outcome in an animal experiment using a restraint stress paradigm which induces depression-like behavioural changes. A dose of etanercept of 0.3 mg/kg body weight was chosen on the base of various studies at rodents in which this compound was effective between 0.15 and 0.8 mg/kg (Inglis et al., 2005; Venegas-Pont et al., 2010; Haugen et al., 2008).

2. Experimental procedures

2.1. Animals

Adult male Wistar rats (outbreed, 12–14 weeks old, $n = 72$, Janvier, Le Genest Saint Isle, France) were housed in standard laboratory cages in groups of four animals for two weeks for acclimatisation. The animals were allowed free access to food and water under a 12-h light–dark schedule (lights on 7:00 a.m.–7:00 p.m.). The experiments were approved by the Animal Welfare Office (Leipzig, Germany; TVV10/11) according to the German guidelines for the use of animals in biomedical research. All efforts were made to minimize the number of animals used and their suffering.

2.2. Drug application

Animals of a body weight of 371 ± 4 g were randomly assigned to six experimental groups ($n = 12$ each). The first group (1) was group housed and served as healthy "naïve" control without restraint or treatment. The animals of the other groups were single housed: (2) no restraint, treatment with Ringer solution (2 ml/kg i.p.) daily, (3) no restraint, treatment with Etanercept (0.3 mg/kg i.p.) instead of Ringer twice a week, (4) restraint, treatment with Ringer solution (2 ml/kg i.p.) daily, (5) restraint, treatment with imipramine (10 mg/kg i.p.) daily, (6) restraint, treatment with Etanercept (0.3 mg/kg i.p.) instead of Ringer twice a week.

Etanercept (Enbrel®) (Pfizer Pharma GmbH, Berlin, Germany) was suspended according to the manufactures instructions and further diluted in Ringer solution. Etanercept and imipramine